Hypothesis

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May spina bifida result from an X-linked defect in a selective abortion mechanism?

SUMMARY It is suggested that the major genetic factor in determining the birth of children with neural tube defects may be a single X-linked gene. It acts as an X-linked dominant, not by producing neural tube defects, but by enabling the affected fetus to survive selective spontaneous abortion. This mechanism, mediated at the deciduo-placental junction, may be under the control of both maternal and fetal genes. With more mutant alleles, survival would become more likely, reaching a maximum in the homozygous affected female fetus of a homozygous affected mother. The female excess in anencephaly is greater than that in spina bifida because of its prenatal severity, thus requiring relatively more mutant alleles for survival.

Background

While the aetiology of neural tube defects is still not fully understood, their occurrence may reasonably be explained by the interaction of environmental factors with genetic predisposition, the multifactorial model (Carter, 1970). Both Carter (1976) and a recent review (Fraser, 1976) have emphasised that the genetic component may be represented by a few loci, or possibly even a single locus.

A striking observation in the epidemiology of neural tube defects is the marked female excess among patients in high risk populations (Carter, 1974), raising the possibility of a single X-linked gene, dominant in effect, determining the birth of babies with neural tube defects. A normal example of such an X-linked dominant is the Xg blood group (Race, 1971).

There is considerable support for an X-linked locus. The high risk of neural tube defects among sibs of an affected child also occurs in maternal half sibs, that is, those with a different father (Carter, 1973), the 1 in 100 risk among cousins is doubled for mothers' sisters' offspring (Yen and MacMahon, 1968), and the high risk in the offspring of north-west European unions is maintained in those of mixed marriages where the father is from the 'low risk' Negro ethnic group (Leck, 1972). Family studies have shown an excessive 'matrilineal' transmission, the basis of the 'plasmagene' theory (Nance, 1971), and Knox (1970) has shown the mathematical possibility of an X-linked locus in the 'fetal-fetal interaction' theory.

It has been estimated in Japan (Nishimura, 1970) that 90% of neural tube defects abort and an estimate for England is 60% (Creasy and Alberman, 1976a). In the valleys of South Wales there exists an inverse relationship between the incidence of spontaneous abortion and spina bifida, suggesting that the survival of such fetuses represents a failure of selective elimination (Roberts and Lloyd, 1973).

These separate approaches raise the possibility of a selective elimination mechanism, controlled by a single locus on the X chromosome, which induces placental separation from the uterine wall in the presence of a neural tube defect. In populations with a high incidence of neural tube defects, a mutant allele is postulated, an 'inborn error of metabolism' which fails to induce the abortion of fetuses with spina bifida or anencephaly and is expressed as an X-linked dominant.

This theory alone can account for a female excess of up to 2 to 1. However, while Carter and Evans (1973a) found a female/male ratio in London of 1·27 to 1 for spina bifida, the ratio for anencephaly was 2·44 to 1. In 'epidemics' of neural tube defects, this female excess rises markedly (Rogers and Morris, 1973) to the extreme of the Birmingham anencephaly epidemic in the 1950s, which was confined to females (Leck, 1972). Clearly, such variation is incompatible with the simple model outlined, and, at the least, an additional locus would seem necessary. A more attractive alternative is offered by the precedent of myotonic dystrophy, normally a relatively mild autosomal dominant disorder, which presents occasionally in a severe congenital form. Harper (1975) found this condition to be confined to fetuses who inherited the gene from their mother, an apparent fetomaternal interaction. Such an interaction of the X-linked loci would result in the genetic predisposition of neural tube defects being controlled by 4 X chromosomes, or 3 if the fetus were male. In short, it offers a 'polyallelic' model instead of a polygenic one.

Normal allele A has gene frequency of 1-f

Abnormal allele a has gene frequency of f

Therefore genotypes AY aY AA Aa aa

have population frequencies $1-f$ f $(1-f)^2$ $2f(1-f)$ f^2

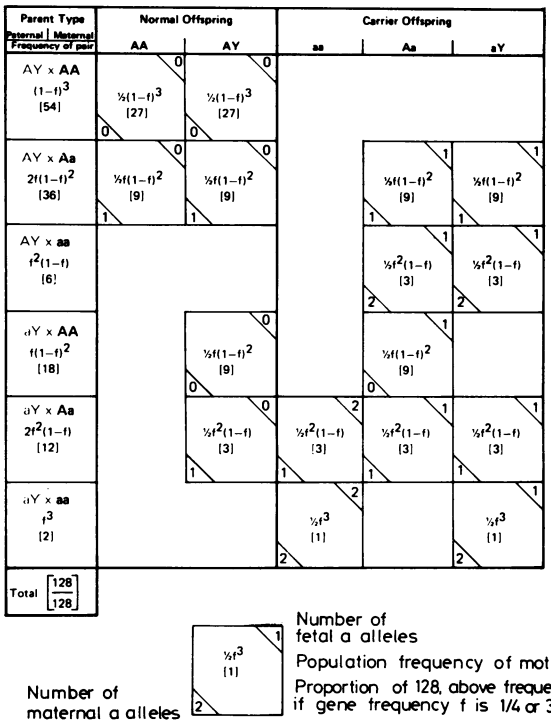

Fig. *Mating types and segregation ratios in their offspring. For illustration, a numerical example is given below where gene frequency f = 0·25.*

EXAMPLE

Sex ratio of survivors if all those with

1*a* allele survive $= \frac{♀\,[9+9+9+3+3+3+1]}{♂\,[9+9+3+3+3+1]} \simeq 1{\cdot}32$ to 1

2*a* $♀/♂ = \frac{[19]}{[16]} \simeq 1{\cdot}19$ to 1

3*a* $♀/♂ = \frac{[7]}{[4]} = 1{\cdot}75$ to 1

4*a* $♀/♂ = \frac{[1]}{[0]}$ = All female

Mathematical model

The Fig. shows the mathematical derivation. The Hardy Weinberg principle is assumed to apply, since subjects with neural tube defects effectively have a reproductive fitness of zero, whether or not they survive to term. Initially, the alleles are assumed to be of equal effect and to combine additively.

Since X chromosome inactivation (lyonisation) affects the placental cells randomly, it should not alter the genetic expression or its analysis. Indeed, if the original analogy is extended, this locus may be close to the Xg blood group which is thought to remain active on both X chromosomes (Race, 1971).

If the mutant allele *a* occurs in the population with a frequency f, and the normal allele *A* occurs with a frequency 1-f, the two male genotypes aY and AY will occur with frequencies f and 1-f, while the female genotypes AA, Aa, and aa will have population frequencies of $(1-f)^2$, $2f(1-f)$, and f^2, respectively. The frequency with which each of the 6 possible mating pairs occurs is obtained by simple multiplication (Fig.). The 16 'fetomaternal' units have population frequencies derived, as shown, by simple division. Also shown is the number of mutant alleles active in each fetomaternal unit. The fraction of the population that each of the 16 units would represent if the gene frequency were 0·25 is included under the Fig.

If the survival to birth of fetuses with spina bifida

depended on the presence of at least one mutant allele, the sex ratio at birth would be 1·32 to 1 at f = 0·25. With a 'threshold' at 2 *a* alleles there would be 1·19 females to each male, at 3 *a* alleles 1·75 to 1, and if 4 *a* alleles were necessary to permit survival to birth the babies would be all female.

This would be likely to give many more posterior neuropore defects when, in fact, the ratio of anterior to posterior defects is about 0·9 at birth. A plausible explanation is that the thresholds are not 'all or none', but graded, with the chance of survival of a defective fetus rising with the number of *a* alleles. Thus, for example, if 25% survive with one allele, 50% with two, 75% with three, and all with four, the sex ratio is 1·333 to 1, independent of gene frequency. Applying this as before to a gene frequency of 0·25, the 2 *a* and 3 *a* thresholds have female/male ratios of 1·5 to 1 and 2·0 to 1, respectively, while the 4 *a* group is all female.

Spina bifida fetuses, with their greater prenatal viability, would require one *a* allele to survive to birth, giving them a postnatal sex ratio of 1·33 to 1 which would remain stable in time and place. 'Anencephalics' with their variable viability would require from one to four *a* alleles for survival and their greater female excess at birth would be particularly marked among the more severely deformed. These predictions correlate closely with previous observations (Leck, 1972, 1974; Rogers and Morris, 1973). The 'all female' epidemic may have been a group of anencephalics so severely deformed that only the presence of four *a* alleles could permit survival to birth.

Thus, in short, the female excess among babies with neuropore defects would be expected to rise with the gene frequency and with the clinical severity of the defect.

Discussion

Male to male transmission represents an important test of any X-linked postulate. To date no such case has been reported, though subfertility in affected males means the number of informative families is as yet small (Carter and Evans, 1973b). If the mutant gene is common, the female carrier rate will be high, with the result that cases of apparent male-to-male transmission would result from unrecognised carrier status in the mother. A knowledge of the incidence of such cases would give an estimate of gene frequency.

If differential prenatal loss is the primary explanation for the female excess, an equivalent male excess is to be expected among abortuses. Polani (1959) found this to be the case in a group of 6 anencephalic fetuses, all of which were apparently male. A second study (Creasy and Alberman, 1976a) examined 10 cases; of the 8 with anterior neuropore defects, 5 were male and 3 were female.

In mathematical terms, this theory closely resembles the 'plasmagene' theory (Nance, 1971) and the 'fetal-fetal interaction' theory (Knox, 1970), while having the advantage of a more plausible biological basis.

Janerich (1975) has proposed the placenta as a target of environmental influence in neural tube defects. He refers to the observation (Scott *et al.*, 1972) that acetazolamide, which on rare occasions causes exencephaly in rats, appears to exert its teratogenic influence on the placenta and produces the malformation more frequently in females. Janerich (1975) suggested that the hormone human chorionic gonadotrophin might be causally related to neural tube defects.

One of us (DG) has studied the hormonal control of labour, and feels that recent evidence is in favour of prostaglandins being the final common path of placental separation. These hormones are effective in causing myometrial contraction and abortion at any time during gestation (Toppozada *et al.*, 1972), while, conversely, their inhibition blocks myometrial activity (Vane and Williams, 1972). Prostaglandin synthesis may depend on lysosomes (Gustavii, 1973) and these are very plentiful in the decidua at the feto-maternal junction (Liggins and Grieves, 1971). Any trauma to these vesicles, particularly from hormonal imbalance, leads to prostaglandin release (Bitensky and Cohen, 1965). It is tempting to speculate on the proposed locus being involved in the prostaglandin metabolic pathway; if so, there is a case for it having an inhibitory role. Compared to the numerical changes in autosomes, there is a disproportionate loss of fetuses with the mild XO karyotype, while an unusually large number of XXX fetuses survive to birth (Creasy and Alberman, 1976b). An excess of prostaglandin inhibitor would normally be repressed in the presence of a neural tube defect. The mutant allele, then, would code for an inhibitor resistant to such repression. This may be why anencephalics, which lack those tissues considered essential to prenatal survival, not only reach term but actually go postmature if labour is not precipitated by hydramnios (Anderson *et al.*, 1972).

A high miscarriage rate before the birth of a child with a neural tube defect has recently been described (Clarke *et al.*, 1975; Laurence and Roberts, 1977). Rather than being causative, this observation probably reflects a negative birth order effect, like that seen in children surviving with neural tube defects (James, 1978). The suggestion is made that these lost conceptions also had spina bifida or anencephaly. An early study (Coffey and Jessop,

1958) described a high overall miscarriage rate in these families, while in the 'high risk' valleys of South Wales there was an inverse relationship between the incidence of spina bifida and the abortion rate (Roberts and Lloyd, 1973). These findings can be accommodated within the present hypothesis by suggesting that environmental teratogens delay neuropore closure in a large group of conceptions, particularly in primiparous women. The great majority abort spontaneously, though in 'high risk' populations the defective *a* gene results in significantly more surviving to term, with an equivalent reduction in fetal loss. The miscarriage rate may appear high in the latter group, either because the control group has had a lesser teratogenic influence and hence fewer abnormal conceptuses with which to contend, or because in some, the *a* alleles delay, but do not prevent, abortion. This would result in relatively more late abortions which are more easily remembered by the mother.

The present hypothesis is able to account for the diverse experimental data relating to neural tube defects which have accumulated. It agrees in essence with most existing theories and is an extension of the multifactorial model. It is amenable to analysis by family studies and linkage analysis, and, with the advent of screening techniques, it may help to identify that group of women at risk of producing a child with spina bifida.

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References

Anderson, A. B. M., Pierrepoint, C. G., Griffiths, K., and Turnbull, A. C. (1972). Steroid metabolism in the adrenals of fetal sheep in relation to natural and corticotrophin induced parturition. *Journal of Reproduction and Fertility*, **16**, (Suppl.), 25–38.

Bitensky, L., and Cohen, S. J. (1965). The variation of endometrial acid phosphatase activity with the menstrual cycle. *Journal of Obstetrics and Gynaecology of the British Commonwealth*, **72**, 769–774.

Carter, C. O. (1970). *Congenital Malformations*. Ed. by F. C. Fraser and V. A. McKusick. Excerpta Medica, Amsterdam.

Carter, C. O. (1973). Spina bifida and anencephalus in Greater London. *Journal of Medical Genetics*, **10**, 209–234.

Carter, C. O. (1974). Clues to the aetiology of neural tube defects. *Developmental Medicine and Child Neurology*, **16**, Suppl. 32, 3–15.

Carter, C. O. (1976). Genetics of common single malformations. *British Medical Bulletin*, **32**, 21–26.

Carter, C. O., and Evans, K. (1973a). Spina bifida and anencephalus in Greater London. *Journal of Medical Genetics*, **10**, 209–234.

Carter, C. O., and Evans, K. (1973b). Children of adult survivors with spina bifida cystica. *Lancet*, **2**, 924–926.

Clarke, C., Hobson, D., McKendrick, O. H., Rogers, S. C., and Sheppard, P. M. (1975). Spina bifida and anencephaly: miscarriage as a possible cause. *British Medical Journal*, **4**, 743–746.

Coffey, V. P., and Jessop, W. J. E. (1958). A three year study of anencephaly in Dublin. *Irish Journal of Medical Science*, **393**, 391–413.

Creasy, M. R., and Alberman, E. D. (1976a). Congenital malformations of the central nervous system in spontaneous abortions. *Journal of Medical Genetics*, **13**, 9–16.

Creasy, M. R., and Alberman, E. D. (1976b). 1st Meeting Clinical Genetics Society, London.

Fraser, F. C. (1976). The multifactorial threshold concept—uses and misuses. *Teratology*, **14**, 267–280.

Gustavii, B. (1973). Studies on the mode of action of injected hypertonic saline in therapeutic abortion. *Acta Obstetricia et Gynecologica Scandinavica*, **25**, (Suppl.), 1–22.

Harper, P. S. (1975). Congenital myotonic dystrophy. II. Genetic basis. *Archives of Disease in Childhood*, **50**, 514–521.

James, W. H. (1978). Birth ranks of spontaneous abortions in sibships of children affected by anencephaly or spina bifida. *British Medical Journal*, **1**, 72–73.

Janerich, D. T. (1975). Female excess in anencephaly and spina bifida: possible gestational influences. *American Journal of Epidemiology*, **101**, 70–76.

Knox, E. G. (1970). Twins and neural tube defects. *British Journal of Preventive and Social Medicine*, **28**, 73–84.

Laurence, K. M., and Roberts, C. J. (1977). Spina bifida and anencephaly: are miscarriages a possible cause? *British Medical Journal*, **2**, 361–363.

Leck, I. (1972). The etiology of human malformations: insights from epidemiology. *Teratology*, **5**, 303.

Leck, I. (1974). Causation of neural tube defects. *British Medical Bulletin*, **30**, 158–162.

Liggins, G. C., and Grieves, S. A. (1971). Possible role for prostaglandin F2α in parturition in sheep. *Nature*, **232**, 629–631.

Nance, W. E. (1971). Anencephaly and spina bifida: an etiologic hypothesis. *Birth Defects: Original Article Series*, **VII**, No. 1, 97–102. The National Foundation—March of Dimes, New York.

Nishimura, H. (1970). Incidence of malformations in abortions. In *Congenital Malformations*, p. 275. Ed. by F. C. Fraser and V. A. McKusick. Excerpta Medica, Amsterdam.

Polani, P. E. (1959). Nuclear chromatin of anencephalic fetuses. *Lancet*, **2**, 240–241.

Race, R. (1971). Human genetics. *Proceedings of the 4th International Congress on Human Genetics, Paris, 1971*.

Roberts, C. J., and Lloyd, S. (1973). Area differences in spontaneous abortion rates in South Wales and their relation to neural tube defect incidence. *British Medical Journal*, **4**, 20–22.

Rogers, S. C., and Morris, M. (1973). Anencephalus: a changing sex ratio. *British Journal of Preventive and Social Medicine*, **27**, 81–84.

Scott, W. J., Butcher, R. E., Kindt, C. W., *et al.* (1972). Greater sensitivity of female than male rat embryos to acetazolamide teratogenicity. *Teratology*, **6**, 239.

Toppozada, M., Bygdeman, M., and Wiqvist, N. (1972). Prostaglandin administration for induction of mid-trimester abortion in complicated pregnancies. *Lancet*, **2**, 1420–1421.

Vane, J. R., and Williams, K. I. (1972). Prostaglandin production contributes to the contractions of the rat isolated uterus. *British Journal of Pharmacology and Chemotherapy*, **45**, 146P.

Yen, S., and MacMahon, B. (1968). Genetics of anencephaly and spina bifida. *Lancet*, **2**, 623–636.

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